

Isoflavone Levels in Five Soybean (*Glycine max*) Genotypes Are Altered by Phytochrome-Mediated Light Treatments

ARA KIRAKOSYAN,[†] PETER KAUFMAN,^{*,†} RANDALL L. NELSON,[‡]
MICHAEL J. KASPERBAUER,[†] JAMES A. DUKE,[§] ELISABETH SEYMOUR,[†]
SOO CHUL CHANG,^{||} SARA WARBER,[†] AND STEVEN BOLLING[†]

University of Michigan Integrative Medicine Program (MIM), University of Michigan, Ann Arbor, Michigan 48104, Department of Crop Sciences, Soybean/Maize Germplasm, Pathology, and Genetics Research Unit, Agricultural Research Service, U.S. Department of Agriculture, University of Illinois, 1101 West Peabody Drive, Urbana, Illinois 61801, Herbal Vineyard, 8210 Murphy Road, Fulton, Maryland 20759, and University College, Yonsei University, Seoul 120-749, Korea

The objective of the present study was to determine whether concentrations of different isoflavones (puerarin, genistein, genistin, daidzein, and daidzin) in shoots and roots of five selected soybean genotypes would respond the same or differently to red (650 nm peak transmittance) and far-red (750 nm peak transmittance) light treatments given under controlled environments. Levels of isoflavones (mg g⁻¹ dry weight biomass) present in seeds, control roots, and shoots and 10 day light-treated seedlings (light, dark, red, and far-red wavelengths) of soybean (*Glycine max*) were determined by high-performance liquid chromatography analysis in comparison with known isoflavone standards. Seeds of the five soybean genotypes studied consistently stored most of their isoflavones as glucosyl conjugates (e.g., daidzin, genistin, and puerarin). For the five soybean genotypes, isoflavone levels were lower in the seeds as compared with roots plus shoots of control, time zero (first true leaf stage) seedlings. Following 10 days of the respective light treatments, we found that (i) isoflavone levels were enhanced in dark-grown plants over light-grown plants for three of the five genotypes (a new finding) and the reverse occurred for a single genotype (a typical response of legumes) and (ii) generally, far-red end of day (EOD) light treatment enhanced total isoflavone levels in roots plus shoots over red EOD light treatment. Results from the present study show that phytochrome does appear to play a role in regulating isoflavone levels in developing soybean seedlings and that this influence by red/far-red-mediated phytochrome reactions is strongly dependent on the genotypes selected for study.

KEYWORDS: *Glycine max*; soybean; isoflavones; red/far-red-mediated phytochrome reactions

INTRODUCTION

Among edible legumes, seeds and seedlings of soybean (*Glycine max*) contain relatively high levels of isoflavones (1–3). Most of the isoflavones are stored as malonyl and glucosyl conjugates in the seeds, whereas in the developing seedlings, the aglycones predominate. The isoflavone levels in the seeds are of the order of 1/10 those in seedlings, arising mostly as a consequence of enhanced synthesis of isoflavones in the latter (1, 4, 5).

In previous studies performed on 24 cultivars of fava beans (*Vicia faba*) (4, 6), we found great variation in levels of the isoflavones, genistein, genistin, daidzein, and daidzin in both

the seeds and the seedlings. These results imply that genotype can have a major influence on the levels of isoflavones. The same kinds of results have been obtained for isoflavone levels in seeds of different soybean genotypes (2). However, soybeans are grown under a range of environmental conditions. These include light, which is involved in the widely studied photosynthetic process, and photomorphogenesis, which influences allocation and use of the products of photosynthesis. Some recent studies have shown that phytochrome (a naturally occurring red/far-red reversible pigment originally found to influence photocontrol of flowering and seed germination) is involved in various biosynthetic pathways, such as starch and soluble sugar metabolism in potato (*Solanum tuberosum*) (7), gibberellin biosynthesis in *Sorghum* and *Arabidopsis* (8, 9), lycopene (10), and other carotenoids (11, 12), and anthocyanin biosynthesis in fruits of tomato (*Lycopersicon esculentum*) (13); phenylpropanoid metabolism (14); alkaloids and phenolic

[†] University of Michigan.

[‡] U.S. Department of Agriculture.

[§] Herbal Vineyard.

^{||} Yonsei University.

compounds of tobacco (*Nicotiana tabacum*) (15); and in many different signal transduction pathways (16).

Because so little is known about the role of phytochrome in the biosynthesis of soybean isoflavones, we posed this question: Would isoflavone concentrations in different cultivars of soybean all respond to the same red and far-red light manipulations of the natural phytochrome system that exists in the growing plant?

To help answer this question, the objective of the present study was to determine whether concentrations of different isoflavones in several different soybean genotypes would respond the same or differently to red and far-red light treatments given under controlled environments. This basic information will be useful in developing outdoor production systems that utilize light reflected from different-colored soil covers (mulches) to sun-grown plants to enhance specific isoflavones (or other phytochemicals) that may provide health benefits to human consumers. This knowledge has already led to the development of practical applications in the use of reflective red plastic mulch to enhance the growth and yield of tomatoes and other agronomic crops, as documented by Kasperbauer (17, 18). It also has enhanced medical benefits [reduction of low-density lipoprotein cholesterol and incidence of atherosclerosis (19)] because phytochrome-mediated red light treatments also result in significant increases in lycopene levels in developing tomato fruits (9).

Results from the present study show that phytochrome does appear to play a role in regulating isoflavone levels in developing soybean seedlings and that this influence by red/far-red-mediated phytochrome reactions is strongly dependent on the genotypes selected for study.

MATERIALS AND METHODS

Because genetic background is an important consideration in examination of types and amounts of isoflavones in edible legume seeds and seedlings (1, 2, 6), we selected five different genotypes of soybean for the present phytochrome study. Two are cultivars developed by conventional plant breeding techniques, and three are primitive cultivars from China that predate scientific plant breeding. The primitive cultivars were PI 490769 from Hubei Province, PI 567417C from Shanxi Province, and PI 603358A from Jilin Province obtained from the U.S. Department of Agriculture Soybean Germplasm Collection housed at the University of Illinois, Urbana-Champaign. The modern cultivars were Cisne (20) and Ripley (21). These soybean genotypes were selected on the basis of known differences in isoflavone content in the seeds. Samples of seeds of each of the five genotypes of soybean were placed in a -80°C freezer for subsequent extraction and high-performance liquid chromatography (HPLC) analysis of isoflavones.

Plant Culture Conditions. Dry seeds (same lots of seeds used for isoflavone analyses) were planted in Perlite in styrofoam plastic cups provided with three 2 mm diameter holes for drainage, five seeds per cup, 12 replicates per genotype. They were germinated at 25°C under continuous illumination with full spectrum lamps providing $600\ \mu\text{E m}^{-2}\ \text{s}^{-1}$ light intensity at the level of the plants. They were watered every 2 days using tap water. At the hook opening stage (4 days later), plants were thinned to two seedlings per cup. When the plants had fully expanded first sets of true leaves and emerging later-formed leaves at the shoot tips (18 day old seedlings), the different light/dark red/far-red light treatments were started.

Environmental Treatments. Seedlings with first true leaves expanded were placed in Precision Scientific Dual Program Illuminated Incubators (no. 818) set at 25°C . The light intensity was $200\ \mu\text{E m}^{-2}\ \text{s}^{-1}$ at the level of the plants. The light source consisted of two full spectrum fluorescent lamps mounted in the incubator door. At the same time, samples of roots and shoots were separately collected for each of the five soybean genotypes and placed in a -80°C freezer for subsequent HPLC analysis of the isoflavones.

The different treatments in the incubators were as follows: (i) continuous dark control for 10 days; (ii) light/dark control (6 h of light/6 h of dark/6 h of light/6 h of dark every 24 h cycle programmed with timers mounted on the incubators); (iii) the same as treatment ii except that 10 min of red light (650 nm) was given at end of every light period; and (iv) the same as treatment ii except that 10 min of far-red light (750 nm) was given at the end of every light period (end of day or EOD). Plants were watered every 2 days. After 10 days of the above treatments, samples of roots and shoots were separately collected for each of the five soybean genotypes, frozen, and placed in a -80°C freezer for subsequent HPLC isoflavone analyses.

Description of Apparatus Used To Expose Soybean Plants to Red and Far-Red Light EOD Treatments. The red (Hoya Red 25A, 58 mm diameter) and far-red (Hoya Infrared R72, 58 mm diameter) filters used in these experiments were obtained from Edmund Optics America, Barrington, NJ. The filters were manufactured by Tokina Co., Ltd. (Japan). Two two-tiered lightweight aluminum chambers were designed and constructed by Charles Roehm and Dennis Kayner (Orthopaedic Surgery Section of the University of Michigan Medical School) for the red/far-red light experiments. The upper chamber ($30\ \text{cm}^2 \times 23\ \text{cm}$ height) was painted with flat black paint inside and contained the light source that was mounted in the center of the chamber ceiling. The light filter was inserted tightly in an opening provided at the center of the basal wall of the chamber. The light source used for the red light 10 min pulse treatments was a Philips 15 W low-energy fluorescent lamp. The light source for the far-red light 10 min pulse treatments was a General Electric 75 W incandescent lamp. The lower chamber ($30\ \text{cm}^2 \times 23\ \text{cm}$ height) was also painted with flat black paint inside. This lower chamber was open at one side so that light from the two fluorescent lamps mounted in the incubator door could reach the plants arranged inside this chamber. When a 10 min pulse of EOD red light or far-red light was required, the upper chamber lamps were turned on by a special timer that was activated when the two door-mounted incubator lamps turned off at the end of each 6 h light photoperiod.

Extraction and HPLC Analysis of Isoflavones. All plant materials (seeds, roots, and shoots of seedlings) were freeze-dried for 48 h with a Labconco lyophilizer. Dry samples were then powdered in a mortar with pestle. Triplicate samples, 0.5 g each, of the fine powder for each experiment were prepared. These samples were extracted with 10 mL of 80% methanol at 60°C for 12 h, and an aliquot (10 μL) of the extract was analyzed by HPLC. The HPLC conditions were as follows: a Phenomenex Luna column (Torrance, CA) (5 μm pore size, C-18, $150\ \text{mm} \times 4.60\ \text{mm}$) with a flow rate of 1 mL/min; solvent A = water + 0.1% TFA (trifluoroacetic acid); solvent B = acetonitrile + 0.1% TFA; HPLC running conditions consisted of a gradient of 5–100% B during a 30 min period; oven temperature, 40°C . The quantitative analysis of each compound in the extracts was analyzed with a Shimadzu HPLC equipped with a photodiode array detector (Shimadzu 10 AD HPLC system from Shimadzu, Japan) to measure the quantities of known isoflavonoids on the basis of the peak height of UV absorption at 280 nm in comparison with peak heights for respective authentic samples. Each peak was identified by the retention time and the characteristic UV spectrum in comparison with the corresponding authentic samples of daidzein, daidzin, genistein, genistin, and puerarin, which were obtained from Sigma-Aldrich Chemical Co. (Milwaukee, WI).

Statistical Analysis of Data. Experiments were repeated at least three times, and the data were analyzed statistically. All results are given as means \pm standard deviation (SD). Differences between variables were tested for significance by Student's *t*-test. A *p* value of <0.05 was considered to be significant.

RESULTS

Analysis of Types and Amounts of Isoflavones Stored in Seeds as Compared with Seedlings of Five Soybean Genotypes. Tables 1–5 show that seeds of the five soybean genotypes studied consistently stored most of their isoflavones as glucosyl conjugates (e.g., daidzin, genistin, and puerarin). Levels of the aglycones, genistein, and daidzein were signifi-

Table 1. Levels of Isoflavones (mg g⁻¹ Dry Weight Biomass) Present in Seeds, Control Roots, and Shoots and 10 Day Light-Treated Seedlings (Light, Dark, Red, and Far-Red Wavelengths) of Soybean (*G. max*) PI 567417C^a

PI 567417C	puerarin	genistein	daidzein	genistin	daidzin	total isoflavones
seeds control	0.150 ± 0.025	ND	0.020 ± 0.035	0.131 ± 0.017	0.124 ± 0.008	0.425
shoots control	1.688 ± 0.233	0.110 ± 0.007	0.082 ± 0.009	0.110 ± 0.088	0.136 ± 0.011	2.126
roots control	0.280 ± 0.034	0.912 ± 0.076	5.618 ± 0.333	ND	0.112 ± 0.016	6.922
shoots light	0.281 ± 0.017	ND	0.013 ± 0.002	0.055 ± 0.004	0.034 ± 0.002	0.383
shoots dark	3.687 ± 0.457	0.038 ± 0.001	0.036 ± 0.002	0.630 ± 0.055	0.036 ± 0.004	4.427
shoots red	1.647 ± 0.233	0.059 ± 0.009	0.036 ± 0.008	0.125 ± 0.032	0.087 ± 0.006	1.954
shoots far-red	2.004 ± 0.312	0.060 ± 0.009	0.051 ± 0.006	0.034 ± 0.003	0.066 ± 0.008	2.215
roots light	0.695 ± 0.078	0.517 ± 0.061	2.297 ± 0.167	0.066 ± 0.005	0.377 ± 0.006	3.952
roots dark	0.947 ± 0.078	0.716 ± 0.092	3.856 ± 0.223	0.041 ± 0.002	0.362 ± 0.004	5.922
roots red	0.452 ± 0.054	0.138 ± 0.024	0.007 ± 0.001	2.840 ± 0.431	0.714 ± 0.065	4.151
roots far-red	0.302 ± 0.022	0.462 ± 0.033	2.315 ± 0.314	0.006 ± 0.001	0.089 ± 0.004	3.174

^a Mean ± SD; *n* = 3; ND, not detected.**Table 2.** Levels of Isoflavones (mg g⁻¹ Dry Weight Biomass) Present in Seeds, Control Roots, and Shoots and 10 Day Light-Treated Seedlings (Light, Dark, Red, and Far-Red Wavelengths) of Soybean (*G. max*) PI 490769^a

PI 490769	puerarin	genistein	daidzein	genistin	daidzin	total isoflavones
seeds control	0.348 ± 0.042	0.022 ± 0.005	0.027 ± 0.006	1.670 ± 0.234	2.094 ± 0.323	4.161
shoots control	1.887 ± 0.222	0.617 ± 0.085	1.121 ± 0.095	0.231 ± 0.041	0.938 ± 0.087	4.794
roots control	0.336 ± 0.051	0.599 ± 0.065	3.531 ± 0.455	ND	0.092 ± 0.008	4.558
shoots light	1.853 ± 0.311	0.354 ± 0.045	0.603 ± 0.075	0.210 ± 0.031	2.308 ± 0.265	5.328
shoots dark	2.295 ± 0.275	0.462 ± 0.051	0.756 ± 0.081	0.327 ± 0.042	3.908 ± 0.513	7.748
shoots red	0.963 ± 0.087	0.362 ± 0.043	0.542 ± 0.062	0.138 ± 0.023	1.944 ± 0.212	3.949
shoots far red	1.439 ± 0.097	0.375 ± 0.052	0.564 ± 0.073	0.200 ± 0.031	2.050 ± 0.296	4.628
roots light	0.220 ± 0.033	3.752 ± 0.572	1.936 ± 0.297	0.024 ± 0.004	0.106 ± 0.009	6.038
roots dark	1.259 ± 0.222	0.655 ± 0.077	2.332 ± 0.343	0.144 ± 0.034	0.601 ± 0.086	4.991
roots red	0.725 ± 0.087	0.280 ± 0.045	0.557 ± 0.075	0.024 ± 0.005	0.070 ± 0.008	1.656
roots far red	0.302 ± 0.041	0.462 ± 0.052	2.315 ± 0.376	0.006 ± 0.001	0.089 ± 0.009	3.174

^a Mean ± SD; *n* = 3; ND, not detected.**Table 3.** Levels of Isoflavones (mg g⁻¹ Dry Weight Biomass) Present in Seeds, Control Roots, and Shoots and 10 Day Light-Treated Seedlings (Light, Dark, Red, and Far-Red Wavelengths) of Soybean (*G. max*) PI 603358A^a

PI 603358A	puerarin	genistein	daidzein	genistin	daidzin	total isoflavones
seeds control	0.121 ± 0.017	ND	ND	0.189 ± 0.021	0.160 ± 0.031	0.470
shoots control	2.439 ± 0.345	0.225 ± 0.033	0.141 ± 0.015	0.423 ± 0.051	0.230 ± 0.032	3.458
roots control	0.334 ± 0.042	0.470 ± 0.055	2.903 ± 0.411	0.019 ± 0.003	0.193 ± 0.032	3.919
shoots light	1.563 ± 0.222	0.053 ± 0.007	0.023 ± 0.004	0.035 ± 0.005	0.119 ± 0.032	1.793
shoots dark	2.624 ± 0.343	0.085 ± 0.009	0.077 ± 0.009	0.162 ± 0.025	0.166 ± 0.032	3.114
shoots red	2.682 ± 0.412	0.087 ± 0.011	0.078 ± 0.009	0.105 ± 0.023	0.146 ± 0.034	3.098
shoots far red	2.750 ± 0.345	0.099 ± 0.012	0.058 ± 0.007	0.060 ± 0.009	0.144 ± 0.025	3.111
roots light	0.609 ± 0.085	0.416 ± 0.055	1.513 ± 0.187	0.036 ± 0.008	0.141 ± 0.033	2.715
roots dark	1.161 ± 0.222	0.282 ± 0.044	0.694 ± 0.087	0.008 ± 0.002	0.089 ± 0.012	2.234
roots red	0.730 ± 0.087	0.429 ± 0.055	1.163 ± 0.097	0.036 ± 0.008	0.164 ± 0.033	2.522
roots far-red	1.089 ± 0.204	0.436 ± 0.054	1.109 ± 0.178	0.121 ± 0.023	0.398 ± 0.045	3.153

^a Mean ± SD; *n* = 3; ND, not detected.

cantly lower. These results are consistent with our previous findings (1). The levels of total isoflavones in the seeds of the five soybean genotypes varied considerably: PI 567417C, 0.425; PI 490769, 4.161; PI 603358A, 0.470; Cisne, 2.393; and Ripley, 6.115 mg gm⁻¹ dry wt.

For the five soybean genotypes, isoflavone levels were significantly lower in the seeds as compared with roots + shoots of T-0 (18 day old) seedlings. They were of the order of 1/10 in PI 567417C, 1/18 in PI 603358A, and 1/2 in PI 490769, Cisne, and Ripley seeds (Tables 1–5). These findings agree with our 1997 data (1). The differences can be accounted for by a combination of enhanced isoflavone synthesis plus release of aglycones from stored conjugates by action of β -glucosidase in the developing seedlings (1).

In T-0 seedlings of the five soybean genotypes, roots consistently produced more daidzein and genistein than shoots in four out of five genotypes (Tables 1–5). In the three primitive

cultivars, puerarin levels were significantly higher in shoots than in roots. In Cisne, the reverse was true for puerarin—more in roots than in shoots. In Ripley, the levels of puerarin in roots and shoots of T-0 seedlings were approximately the same.

Comparison of Isoflavone Levels in Seedlings of Five Soybean Genotypes Following a Succession of Light/Dark vs Dark Treatments Over a 10 Day Period. In comparing results for light vs dark treatments in Tables 1–5, we see that after 10 days of such treatments in the incubators, isoflavone levels for shoots and roots were significantly greater in dark-grown seedlings than in light-grown seedlings for PI 567417C (ca. two times greater), for PI 603358A (ca. 1.25 times greater), and for Cisne (ca. two times greater). In PI 490769, they were essentially the same. In Ripley, the isoflavone levels were greater in light-grown seedlings by a factor of 1.3. The light vs dark effects that we see for Ripley are typical for other edible legume seedlings that we have tested (1, 4–6). The opposite results

Table 4. Levels of Isoflavones (mg g⁻¹ Dry Weight Biomass) Present in Seeds, Control Roots, and Shoots and 10 Day Light-Treated Seedlings (Light, Dark, Red, and Far-Red Wavelengths) of Soybean (*G. max*) Cultivar Cisne^a

Cisne	puerarin	genistein	daidzein	genistin	daidzin	total isoflavones
seeds control	0.197 ± 0.031	ND	0.014 ± 0.003	1.285 ± 0.097	0.897 ± 0.078	2.393
shoots control	0.003 ± 0.001	0.096 ± 0.008	ND	ND	ND	0.099
roots control	1.571 ± 0.253	0.376 ± 0.056	2.525 ± 0.355	0.037 ± 0.007	0.188 ± 0.043	4.697
shoots light	2.504 ± 0.456	0.069 ± 0.009	0.047 ± 0.008	0.023 ± 0.006	0.355 ± 0.055	2.998
shoots dark	6.627 ± 0.877	0.232 ± 0.045	0.214 ± 0.053	0.276 ± 0.047	0.161 ± 0.022	7.510
shoots red	3.022 ± 0.453	0.169 ± 0.027	0.269 ± 0.045	0.564 ± 0.076	0.428 ± 0.055	4.452
shoots far red	3.030 ± 0.455	0.511 ± 0.067	0.488 ± 0.075	1.158 ± 0.333	0.419 ± 0.056	5.606
roots light	0.894 ± 0.095	0.363 ± 0.045	0.946 ± 0.087	0.020 ± 0.007	0.077 ± 0.009	2.300
roots dark	1.562 ± 0.321	0.354 ± 0.055	0.511 ± 0.066	0.094 ± 0.005	0.099 ± 0.009	2.540
roots red	0.919 ± 0.088	0.352 ± 0.054	0.661 ± 0.077	0.021 ± 0.007	0.058 ± 0.009	2.011
roots far-red	0.671 ± 0.088	0.476 ± 0.056	3.154 ± 0.443	ND	0.114 ± 0.022	4.4 15

^a Mean ± SD; *n* = 3; ND, not detected.**Table 5.** Levels of Isoflavones (mg g⁻¹ Dry Weight Biomass) Present in Seeds, Control Roots, and Shoots and 10 Day Light-Treated Seedlings (Light, Dark, Red, and Far-Red Wavelengths) of Soybean (*G. max*) Cultivar Ripley^a

Ripley	puerarin	genistein	daidzein	genistin	daidzin	total isoflavones
seeds control	0.192 ± 0.033	0.091 ± 0.008	0.073 ± 0.009	3.126 ± 0.532	2.633 ± 0.411	6.115
shoots control	0.461 ± 0.378	0.132 ± 0.033	0.112 ± 0.025	1.238 ± 0.311	0.477 ± 0.055	2.420
roots control	0.369 ± 0.045	0.803 ± 0.095	6.570 ± 0.788	0.037 ± 0.007	0.314 ± 0.051	8.093
shoots light	2.873 ± 0.357	0.119 ± 0.027	0.049 ± 0.009	0.608 ± 0.085	0.182 ± 0.035	3.831
shoots dark	2.522 ± 0.423	0.268 ± 0.045	0.195 ± 0.027	0.039 ± 0.009	1.097 ± 0.245	4.121
shoots red	1.997 ± 0.035	0.201 ± 0.048	0.178 ± 0.031	1.474 ± 0.178	1.997 ± 0.207	5.847
shoots far red	1.663 ± 0.118	0.215 ± 0.033	0.199 ± 0.029	0.045 ± 0.009	0.966 ± 0.088	3.088
roots light	0.496 ± 0.055	1.004 ± 0.098	4.695 ± 0.521	0.137 ± 0.028	0.598 ± 0.076	6.930
roots dark	1.023 ± 0.099	1.112 ± 0.174	2.997 ± 0.389	0.029 ± 0.345	0.194 ± 0.025	5.355
roots red	0.305 ± 0.045	ND	ND	ND	0.031 ± 0.009	0.336
roots far-red	0.561 ± 0.078	0.633 ± 0.085	1.218 ± 0.186	0.019 ± 0.008	0.081 ± 0.009	2.602

^a Mean ± SD; *n* = 3; ND, not detected.

that were obtained for PI 567417C, PI 603358A, and Cisne are new and very interesting.

Comparison of Isoflavone Levels in Seedlings of Five Soybean Genotypes Following EOD 10 min Exposures to Red vs Far-Red Light Over a 10 Day Period. The phytochrome responses to EOD (actually end of each light period) 10 min exposures to red or far-red light elicited some interesting responses in terms of differences in isoflavone levels in the five soybean genotypes (**Tables 1–5**): (i) In PI 567417C, far-red > red by a factor of ca. 1.25. Both red and far-red treatments enhanced isoflavone levels over those for light per se significantly (by a factor of ca. two times greater). (ii) In PI 490769, far-red > red by a factor of ca. 2.2. As compared with light treatment per se, *R* = ca. 1/2 the levels in light whereas far-red > light by a factor of 1.08. (iii) In PI 603358A, far-red > red by a factor of 1.14. As compared with light treatment per se, both red and far-red treatments > light by factors of 1.24 for red and 1.39 for far-red. (iv) In Cisne, far-red > red by a factor of 1.48. As compared with light treatment per se, both red and far-red treatments > light by factors of 1.22 for red and 1.81 for far-red. (v) In Ripley, far-red ≈ red. Both red and far-red treatments < light by factors of 0.59 for red and 0.65 for far-red.

Our results indicate that there is a phytochrome red/far-red response in soybeans, where the magnitude of response depends on the genotype. Generally, far-red treatment enhanced total isoflavone levels in roots and shoots of soybean seedlings over red treatment and over light treatment in all genotypes except Ripley. However, for Ripley, both red and far-red treatments were significantly < light, and in PI 490769, red treatment was significantly less than light, whereas far-red is greater than light but only by a factor of 1.09.

DISCUSSION

Soybean seeds and seedlings are relatively rich sources of isoflavones (phenylpropanoids) when compared with other edible legumes (1–5). The levels of isoflavones in the seeds varied among the five genotypes that we selected for study (**Tables 1–5**). Similar results were obtained by Lozovaya et al. (2) for seeds of different soybean genotypes whose plants were grown at different temperatures and soil moisture conditions. Again, looking at **Tables 1–5**, we observe that levels of isoflavones in soybean seedlings are generally higher than in seeds, particularly in the root systems. This is because of enhanced synthesis of isoflavones and release of aglycones from isoflavone conjugates stored in the seeds as the soybean seedlings develop (1).

These enhanced isoflavone levels seen in soybean seedlings are triggered by dark (as compared with light) (**Tables 1–5**). However, for the two light treatments, red and far-red phytochrome regulation is evident, where we show that 10 min of far-red light EOD treatments over a 10 day period significantly elevate isoflavone levels in both roots and shoots of the seedlings over comparable red light EOD treatments, particularly in the roots. It is interesting, in this connection, that the extent to which phytochrome is involved in this type of regulation depends a great deal on the genotype being studied. In other plants, light has been shown to upregulate phenylpropanoid biosynthesis in *Arabidopsis* roots (14). In tomato (*Lycopersicon esculentum*) fruits, lycopene levels are elevated up to 2.3-fold by red light treatment, which is reversed by far-red light treatment (10).

The mechanism by which phytochrome-mediated red and far-red light treatments enhance levels of isoflavones in the phenylpropanoid metabolic pathway (see ref 3 for details on the pathway) in plants remains unclear. However, it has been

established that light exposure can lead to changes in expression of genes involved in phenylpropanoid metabolism in *Arabidopsis* roots, some of which are enhanced in their expression and/or activity and could possibly explain enhanced levels of flavonoids and of coniferin and syringin metabolites observed (14). Bauer et al. (22) suggest that light acts by enhancing nuclear import of phytochrome photoreceptors, promotes the interaction of phytochrome A (phyA) and phytochrome B (phyB) with transcription factors (e.g., PIF3, phytochrome interacting factor 3), and triggers a signal transduction cascade that regulates the expression of ca. 2500 genes in *Arabidopsis thaliana*.

Our results may have important implications for soybean plants grown in the field, where solar far-red light flux predominates at sunset (EOD). Mandoli et al. (23) and Tester and Morris (24) report that far-red light and red light can travel downward in loam soil up to 2 and 10 cm in sand. This might be sufficient for such light exposures, even for as short a time as 10 min over a 10 day period, to cause isoflavone production to increase in soybean roots growing in upper layers of the soil. Even more interesting is the fact that plant roots can transmit light along the root axis (like light pipes) to roots occurring at deeper levels in the soil (14, 23, 25). Because the isoflavone, genistein, is a primary receptor molecule for establishment of nitrogen-fixing rhizobacteria in legume roots (3), including soybeans, its elevated levels in light-exposed roots could lead to more favorable nitrogen fixation, enhanced growth of soybean plants, and greater accumulation of soluble proteins in the seeds. Of equal importance is the fact that elevated levels of isoflavones in the roots could lead to enhanced resistance to attack by pathogenic fungi (1).

LITERATURE CITED

- (1) Kaufman, P. B.; Duke, J. A.; Briemann, H.; Boik, J.; Hoyt, J. E. A comparative survey of leguminous plants as sources of the isoflavones, genistein and daidzein: Implications for human nutrition and health. *J. Comp. Alt. Med.* **1997**, *3*, 7–12.
- (2) Lozovaya, V. V.; Lygin, A. Y.; Ulanov, A. V.; Nelson, R. L.; Daydé, J.; Widholm, J. M. Effect of temperature and soil moisture status during seed development on soybean seed isoflavone concentration and composition. *Crop Sci.* **2005**, *45*, 1934–1940.
- (3) Cseke, L. J.; Kirakosyan, A.; Kaufman, P. B.; Warber, S. L.; Duke, J. A.; Briemann, H. L. *Natural Products from Plants*, 2nd ed.; CRC Press-Taylor and Francis Group, LLC: Boca Raton, FL, 2006.
- (4) Kaufman, P. B.; Kirakosyan, A.; Cseke, L. J.; Duke, J. A.; Warber, S.; Bolling, S. F. Biotechnology studies on isoflavone production in edible legumes. *Recent Res. Dev. Plant Sci.* **2005**, *3*, 85–103.
- (5) Lal, A.; Warber, S.; Kirakosyan, A.; Kaufman, P. B.; Duke, J. A. Up-regulation of isoflavonoids and soluble proteins in edible legumes by light and fungal elicitor treatments. *J. Alt. Comp. Med.* **2003**, *9*, 371–378.
- (6) Kirakosyan, A.; Kaufman, P. B.; Duke, J. A.; Warber, S.; Bolling, S. The production of L-dopa and isoflavones in seeds and seedlings of different cultivars of *Vicia faba* L. (fava bean). *Evidence-Based Integr. Med.* **2004**, *1*, 1–5.
- (7) Schittenheim, S.; Menge-Hartmann, U.; Oldenberg, E. Photosynthesis, carbohydrate metabolism, and yield of phytochrome-B-overexpressing potatoes under different light regimes. *Crop Sci.* **2004**, *44*, 131–143.
- (8) Lee, I.-J.; Foster, K. R.; Morgan, P. W. Photoperiod control of gibberellin levels in Sorghum. *Plant Physiol.* **1998**, *116*, 1003–1011.
- (9) Blazquez, M. A.; Weigel, D. Independent regulation of flowering by phytochrome B and gibberellins in *Arabidopsis*. *Plant Physiol.* **1999**, *120*, 1025–1032.
- (10) Alba, R.; Cordonnier-Pratt, M.-M.; Pratt, L. H. Fruit-localized phytochromes regulate lycopene accumulation independently of ethylene production in tomato. *Plant Physiol.* **2000**, *123*, 363–370.
- (11) Khudairi, A. K.; Arboleda, O. P. Phytochrome-mediated carotenoid biosynthesis and its influence by plant hormones. *Physiol. Plant* **1971**, *24*, 18–22.
- (12) Thomas, R. L.; Jen, J. J. Phytochrome-mediated carotenoid biosynthesis in ripening tomatoes. *Plant Physiol.* **1975**, *56*, 452–453.
- (13) Kendrick, R. E.; Kronenberg, G. H. M. *Photomorphogenesis in Plants*, 2nd ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1994.
- (14) Hemm, M. R.; Rider, S. D.; Ogas, J.; Murry, D. J.; Chapple, C. Light induces phenylpropanoid metabolism in *Arabidopsis* roots. *Plant J.* **2004**, *38*, 765–778.
- (15) Tso, T. C.; Kasperbauer, M. J.; Sorokin, T. P. Effect of photoperiod and end-of-day light quality on alkaloids and phenolic compounds of tobacco. *Plant Physiol.* **1970**, *45*, 330–333.
- (16) Uniprot Knowledgebase keyword: Phytochrome signaling pathway. UniProtKB/Swiss-Prot.
- (17) Kasperbauer, M. J. Colored mulch for food crops. *Chemtech.* **1999**, August, 45–50.
- (18) Kasperbauer, M. J.; Loughrin, J. H.; Wang, S. Y. Light reflected from red mulch to ripening strawberries affects aroma, sugar and organic acid concentrations. *Photochem. Photobiol.* **2001**, *74*, 103–107.
- (19) Agarwal, S.; Rao, A. V. Tomato lycopene and low-density lipoprotein oxidation: A human dietary intervention study. *Lipids* **1998**, *33*, 981–984.
- (20) Nickell, C. D.; Thomas, D. J.; Cary, T. R.; Stephens, P. A. Registration of 'Cisne' Soybean. *Crop Sci.* **1996**, *36*, 1410.
- (21) Cooper, R. L.; Martin, R. J.; McBlain, B. A.; Fioritto, R. J.; St. Martin, S. K.; Calip-DuBois, A.; Schmitthenner, A. F. Registration of 'Ripley' soybean. *Crop Sci.* **1990**, *30*, 963.
- (22) Bauer, D.; Viczián, A.; Kircher, S.; Nobis, T.; Nitschke, R.; Kunkel, T.; Panigrahi, K. C. S.; Adam, E.; Fejes, E.; Schäfer, E.; Nagy, F. Constitutive photomorphogenesis 1 and multiple photoreceptors control degradation of phytochrome interacting factor 3, a transcription factor required for light signaling in *Arabidopsis*. *Plant Cell* **2004**, *16*, 1433–1445.
- (23) Mandoli, D. F.; Ford, G. A.; Waldron, L. J.; Nemson, J. A.; Briggs, W. R. Some spectral properties of several soil types—Implications for photomorphogenesis. *Plant Cell Environ.* **1990**, *13*, 287–294.
- (24) Tester, M.; Morris, C. The penetration of light through soil. *Plant Cell Environ.* **1987**, *10*, 281–286.
- (25) Sun, Q.; Yoda, K.; Suzuki, M.; Suzuki, H. Vascular tissue in the stem and roots of woody plants can conduct light. *J. Exp. Bot.* **2003**, *54*, 1627–1635.

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